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phosphatidylserine (PtdSer) at the PM [1]. Previous studies have shown that PDK1 homodimerisation is essential for its regulating activity [2].
Our goal is to elucidate the role of PtdIns(3,4,5)P_3 and PtdSer in the homodimerisation mechanism of PDK1. Measuring Förster Resonance Energy Transfer (FRET) by Fluorescence Lifetime Imaging Microscopy (FLIM) we have studied the spatial and temporal distribution of the homodimeric population of wild-type and point mutants of PDK1 with decreased affinity for PtdIns(3,4,5)P_3 and PtdSer on individual human breast cancer cells (SK-BR-3) and non-cancerous mouse fibroblasts (NIH-3T3) before and after growth factor stimulation. Based on the statistical comparison of these populations, we have established the critical and cooperative role of both phospholipids for the recruitment of this kinase at the PM and its subsequent regulation through the intermolecular organisation of homodimers.
1. Lucas N and Cho W (2011) Phosphatidylserine Binding Is Essential for Plasma Membrane Recruitment and Signaling Function of 3-Phosphoinositide-dependent Kinase-1. J Biol Chem 286: 41265-41272.
2. Masters TA, Calleja V, Armoogum DA et al (2010) Regulation of 3-Phosphoinositide-Dependent Protein Kinase 1 Activity by Homodimerization in Live Cells. Science Signaling 3(145): ra78.

2647-Pos Board B339
HER2 Overexpression Induces Membrane Deformation that Increases Cell Motility
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Approximately 20% of breast cancers (BC) are characterized by the gene amplification and overexpression of HER2, a member of the ErbB/HER receptor family. While targeted therapies against HER2 effectively delay disease progression in this BC subtype, details of how overexpressed HER2s drive these tumors to malignancy are still unclear. To better understand this process, we investigated cellular responses to HER2 overexpression in individual live cells. We developed novel single receptor diffusion analyses to probe the spatial distribution of HER2s and determine their activation status by their diffusivity. Surprisingly, we found that HER2 overexpression induced deformation of basal cell membranes, which depended only on the HER2 density, regardless of the receptor signaling activity. Moreover, this membrane deformation lowered the focal adhesion coverage on the cell surface, which reduced cell adhesion and increased cell motility. These findings suggest that there is a signaling-independent role of HER2 overexpression in disease progression of HER2 positive BCs.

2648-Pos Board B340
CD44-Based Adhesion and Mechanotransductive Signaling on Engineered Hyaluronic Acid Matrices
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Glioblastoma multiforme (GBM) is the most clinically aggressive primary brain tumor and is characterized by diffuse infiltration of glioma cells into brain parenchyma, which is rich in the glycosaminoglycan hyaluronic acid (HA). The extracellular matrix of GBM tumors is associated with aberrant HA secretion, overexpression of the HA receptor CD44, and tissue stiffening. Here we combine matrix engineering and genetic manipulation of GBM tumor cells to determine the role of HA/CD44 adhesion in the outputs of cell adhesion, migration, and invasion. We show that HA/CD44 adhesion contributes to the mechanosensing and invasive motility of GBM tumor cells, both intrinsically and in the context of RGD/integrin adhesion. We also find that three-dimensional cell invasion is maximized in an HA-rich environment, but reduction of CD44-based adhesion or addition of integrin-adhesive peptides to the matrix suppresses invasion. While comparatively much is known about cell-matrix mechanosensing through integrins and the role it may play in tumor progression, our findings reveal a previously under-appreciated role of CD44 and possibly other HA-specific receptors in this process. These findings have broad implications for the field's fundamental understanding of how cancer cells interact with the tumor microenvironment and suggest new therapeutic strategies.

2649-Pos Board B341
Regulation of the HER3/ErbB3 Pseudokinase Domain by an ATP-Competitive Inhibitor
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HER3 (or ErbB3) is a member of the human epidermal growth factor receptor (HER/EGFR) family of receptor tyrosine kinases. HER3 carries inactivating mutations in its kinase domain and therefore is denoted as a pseudokinase. Despite lacking catalytic activity, the pseudokinase domain of HER3 plays an important role in activating EGFR and HER2 kinases, resulting in potent downstream signaling. Recent advancements in cancer research identify HER3 as an important therapeutic target in breast, lung, gastric, ovarian and colorectal cancers, but due to HER3's pseudokinase status, its inhibition remains a major challenge. Our past work demonstrated that the pseudokinase domain of HER3 tightly binds ATP. This finding suggested an exciting opportunity - it might be possible to regulate HER3 signaling through modulation of its nucleotide-binding pocket. However, due to lack of available compounds, this idea was left untested. Here we are reporting that the allosteric activator function of HER3 can be modulated through this site via the ATP-competitive inhibitor bosutinib. We present a first structure of the HER3 pseudokinase domain with an inhibitor, bosutinib, bound in the nucleotide-binding site. To our knowledge, this is the first structure of any pseudokinase bound to an inhibitor. We then perform in vitro assays to show for the first time that HER3 function can be modulated through small molecule binding to the nucleotide-binding pocket. Although we show that bosutinib has the opposite effect from what is desired in cancer therapies, our study provides the proof of principle for small molecule regulation of the HER3 function. This finding opens an exciting direction for the generation of a new class of HER3-specific therapeutics that directly target the pseudokinase domain.

2650-Pos Board B342
Archazolid-B Provides Alternative Therapy for Trastuzumab-Resistant ErbB2 Positive Breast Cancer
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Trastuzumab is the most commonly used humanized monoclonal antibody for specific therapy of breast cancer. Even though its clinical introduction was a breakthrough, a large fraction of the treated tumors are or become resistant. It is well known that receptor internalization and recycling are crucial for ErbB2 mediated signal transduction. Accordingly, inhibition of its molecular machinery could provide alternatives for tumor treatment. Vacuolar H^+-ATPase (V-ATPase) is involved in the regulation of endocytotic/recycling pathways. Archazolid-B was described as a potent V-ATPase inhibitor, which induces apoptosis and impairs migration of tumor cells. Based on these observations we investigated the effect of Archazolid on trastuzumab-resistant Jimt-1 cells *in vitro* and *in vivo*. 10nM of Archazolid caused decreased membrane ErbB2 expression, which was accompanied by the reduced relative phosphorylation on Y1248 and intracellular accumulation of the receptors. As an *in vivo* model, SCID mice were transplanted with Jimt-1 xenografts and treated with Archazolid. Following administration of the V-ATPase inhibitor there was a significant decrease in tumor growth compared to control tumors. Confocal microscopic images of tumor sections showed heterogeneous distribution of the proliferation marker Ki67 positivity. Tumor areas with low Ki67 nuclear expression showed intracellular accumulation of ErbB2 molecules similarly to the *in vitro* experiments, compared to high Ki67 expressing areas with prominent membrane ErbB2 expression. This heterogeneity might be due to diverse tumor-vascularization. Our results demonstrate that Archazolid can be used for *in vitro* and *in vivo* tumor growth inhibition based on its interference with ErbB2 recycling.

Exocytosis and Endocytosis II

2651-Pos Board B343
Rescue of Dopamine Release and Behavior by Transplanted Neural Stem Cells in a Rat Model of Parkinsonism
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Parkinson's disease (PD) is a neurodegenerative disorder due to reduced dopamine (DA) in the striatum and loss of DA neurons in the substantia nigra pars compacta. Embryonic stem cells (ESCs) are an optimal source for cell therapy for PD. We recently developed a fast (one-week) protocol using small molecules that effectively induces human ESCs to become primitive neural stem cells (pNSCs), which are then differentiated into DA-like neurons *in vitro*. As pNSCs are infinitely expandable, this approach offers a strategy to readily generate DA neurons on a large scale. But whether these pNSC-differentiated DA (pNSC-DA) neurons can functionally integrate into the damaged brain is unknown.